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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/582,277	06/10/2006	Allan Nielsen	10527.204-US	1622

25908 7590 03/25/2011

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EXAMINER	
NOAKES, SUZANNE MARIE	
ART UNIT	PAPER NUMBER
1656	

NOTIFICATION DATE	DELIVERY MODE
03/25/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents-US-NY@novozymes.com

Office Action Summary	Application No.	Applicant(s)	
	10/582,277	NIELSEN ET AL.	
	Examiner	Art Unit	
	SUZANNE NOAKES	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 January 2011*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *41 and 46-70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *41 and 46-70* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of the Claims

1. Claims 41 and 46-70 are pending and subject to examination on the merits; claims 66-70 are newly added in the amendments of 01/28/2011.

Withdrawal of Previous Objections/Rejections

2. The objection to clams 47 and 59 are withdrawn in view of the amendments to the claims.

3. The rejection of claims 41, 46-49, 51, 52, 59-61, 63, 64 under 35 U.S.C. 102(b) as being anticipated by Hartford and Dowds (Microbiology, 1994, Vol. 140, pp. 297-304) as evidenced by Chen et al. (Mol. Micro., 1995, cited on IDS) is withdrawn in favor of a modified rejection as recited below, said modification is necessitated by Applicants amendments.

4. The rejection of claims 41, 46-65 under 35 U.S.C. 102(b) as being anticipated by Chen et al. (PNAS - 1995) is withdrawn in favor of a modified rejection as recited below, said modification is necessitated by Applicants amendments.

New Objections/Rejections – Necessitated by Amendments

35 USC § 112 – 2nd paragraph

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 41, 46 and 70 recite the limitation in the last wherein clause of each claim, that the *Bacillus* progeny cell produces great amounts of a secreted or heterologous protein of interest than the *Bacillus* parent strain, in reference to the preamble which states it is a method for enhancing secretion of *any* protein of interest (claim 41), or a method of producing *any* protein of interest (claims 46 and 70), not necessarily one which is secreted or heterologous. Thus, there is insufficient antecedent basis for these limitations in the claims. Claims 48-58 and 60-69 are included in the instant rejection as they do not remedy the noted deficiency.

35 USC § 112 – 4th paragraph

7. The following is a quotation of the fourth paragraph of 35 U.S.C. 112:

> Subject to the following paragraph, a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

8. Claims 49 and 61 are rejected because they recite the limitation "wherein the protein of interest is homologous or heterologous", however, independent claims 41 and 46 require that the protein of interest to be heterologous. Thus, claims 49 and 61 do not further limit the claimed subject matter.

9. Claim 55 is rejected because it recites the limitation wherein the nucleotide sequence has at least 90% identity to SEQ ID NO: 1", which notably encodes SEQ ID NO: 2 of claim 41, however, independent claim 41 requires at least 95% identity to SEQ ID NO: 2 and thus it does not further limit the claimed subject matter

Claim Rejections - 35 USC § 102

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

11. Claims 41, 46-49, 51, 52, 59-61, 63, 64 and new claims 66-69 are rejected under 35 U.S.C. 102(b) as being anticipated by Hartford and Dowds (Microbiology, 1994, Vol. 140, pp. 297-304) as evidenced by Chen et al. (Mol. Micro., 1995, cited on IDS) and by Naclerio et al. (App. Env. Micro., 1995, 61(12):4471-4473).

Hartford and Dowds teach the isolation and characterization of hydrogen peroxide resistant mutant strains from *Bacillus subtilis*. The parent strain YB886 was exposed to increasing concentrations of hydrogen peroxide, several resistant cells survived and resulted in the creation of a new spontaneous progeny strain termed MA991. It is taught that several different proteins were over produced (termed over-accumulated in the reference) as compared to the wild-type parent strain. Specifically, Figure 5 shows the size of the proteins and N-terminal sequences of said proteins which were over-produced in the MA991 strain as compared to the parent strain YB886. It is noted that the 113 kDa protein, although it lacks the N-terminal methionine, is 100% identical to the amino acids 2-16 of the instant SEQ ID NO: 2. The next 10 amino acids, however, are different.

Hartford 113 kDa ? K T E N A K T N Q T L V E N K S T T Q T V F R M H

SEQ ID NO: 2 M K T E N A K T N Q T L V E N S L N T Q L S N W F L

Chen et al. teach using the MA991 strain as taught by Hartford and Dowds to make other progeny strains therefrom.

It is specifically stated (see p. 297, 1st col., 1st paragraph, ½ way down to 2nd col., 1st paragraph):

> "Hartford and Dowds (1994) isolated strain MA991 as a spontaneous H_2O_2-resistant derivative of YB886, which constitutively synthesizes high levels of H_2O_2-inducible proteins. These include catalase (KatA; 59.5 kDa) and alkyl hydroperoxide reductase (AhpC and AhpF; 23 and 53 kDa) (Fig. 4). In addition, MA991 overproduces two proteins with apparent molecular masses of 16 and 113 kDa and is reduced for flagellin (hag) expression.
>
> We noticed that the published amino-terminal sequence of the 113 kDa protein overproduced in MA991 (Hartford and Dowds, 1994) is identical to MrgA for the first 15 of 26 amino acids. To test the relationship between mrgA and this 113 kDa protein, we transformed our mrgA-lacZ fusion into MA991 to generate strain HB1032. In stationary phase, HB1032 still overproduced KatA, AhpC and AhpF, but neither the 16 kDa nor the 113 kDa protein was observed by SDS-PAGE (Fig. 4). This suggests that mrgA is the structural gene for these two observed protein bands; the 113 kDa band presumably represents a stable oligomeric complex (see below) which the 16 kDa band is the appropriate size for the MrgA monomer. In support of this hypothesis, the amino-terminal sequence of the 16 kDa band was subsequently found to match exactly the predicted MrgA sequence (H. Cameron, unpublished data; cited in Dowds, 1994). This suggests that the late cycles of amino acid sequence of the 113 kDa protein were in error.
>
> Previous pulse-labelling studies have identified a 16 kDa protein as the most strongly induced band following treatment of growing B. subtilis cells with 50 μM H_2O_2 (Murphy et al., 1987). Its synthesis is highest during the first 5 to 10 min after treatment with H_2O_2 and returns to the low basal level within 30 min (Dowds et al., 1987) consistent with the transient induction of mrgA-lacZ observed in our gene fusion experiments (Fig. 3). Therefore, we conclude that this 16 kDa band represents the MrgA monomer which then assembles into a stable, oligomeric complex detected as the 113 kDa band by SDS-PAGE."

Therefore, Chen et al. make it sufficiently clear that the 113 kDa protein N-terminal sequence as taught by Hartford and Dowd originally was in error for amino acids 15-26 and evidences that said 113 kDa protein is inherently MrgA in oligomeric complex form. Said 113 kDa complex is clearly overproduced in MA991 as compared to YB886 as taught by Hartford and Dowd – see Figures 3 & 5, as are several other "proteins of interest" (e.g. KatA, AhpC and AhpF), .

It is further noted that step (b) as in claim 46, e.g. "recovering the protein" is met by the isolation of said protein on the noted SDS gels as well as the N-terminal sequencing of said protein complex.

With regard to the limitation that the protein of interest is secreted (or heterolgous), it is noted that Naclerio et al. demonstrate that KatA is a secreted enzyme in *Bacillus subtilis* under hydrogen peroxide inducing conditions, both of which are the same species and conditions utilized by Hartford and Dowd.

Applicants Remarks and Examiner's Rebuttal:

Applicants state that they believe the amended claims overcome the rejection of Hartford and Down as evidenced by Chen et al. but provide no rationale or reasoning in making these assertions.

It is clear from the claims and the specification that the mere presence of a *Bacillus* cell expressing MrgA will enhance the overexpression and secretion of a protein of interest; said MrgA protein is by definition a chaperone protein whose activity is specific for just this purpose. Claims 41, 48-58, 66, 68 and 70 require nothing more

than the overexpression of a single copy of MrgA protein in order for enhanced secretion to take place. Hartford and Dowds as evidenced by Chen et al. clearly teach the overexpression of MrgA in a *Bacillus* progeny cell which results in the overexpression of other proteins of interest such as KatA, AhpC and AhpF. Furthermore, KatA clearly is a *Bacillus* secreted enzyme as shown by Naclerio et al. when produced under conditions such as hydrogen peroxide induction (used by both Hartford and Dowd and also Naclerio et al.) and as such, the KatA and MrgA proteins overproduced and secreted thus meet the limitations of the claims.

With regard to the sequence identities, the claims stand rejected because by all comparable data, the protein isolated by Hartford and Dowds as evidenced by Chen et al. is the same exact protein consisting of instant SEQ ID NO: 2 (e.g. 100% identity) because the genus and species of bacteria are identical for which the protein was isolated because in addition to the N-terminus being identical, the relative molecular weight of the proteins isolated are identical.

12. Claims 41, 46-65 and new claims 66, 67 and 70 are rejected under 35 U.S.C. 102(b) as being anticipated by Chen et al. (1995, PNAS, Vol. 140, pp. 297-304) as evidenced by Naclerio et al. (App. Env. Micro., 1995, 61(12):4471-4473).

Chen et al. teach the creation of various strains of *Bacillus subtilis* which have been transformed with exogenous chromosomal mrgA-lacZ fusion DNA which is then fused to either the mrgA or katA promoters and expressed (see p. 8190 and p. 8192, as

recited below). Said expression results in progeny cells which produce greater amounts of the fusion construct than the parent strain. It is specifically taught:

> Each cured strain (HB13XX series) suspected of carrying a trans-acting mutation (because the constitutive phenotype was not phage-linked) was transduced to resistance to erythromycin and colincomycin by SPβ1122, generating strains HB12XXB, and constitutive expression of β-gal was confirmed. To study the regulation of mrgA and mrgC (1) in these mutant backgrounds, each HB13XX strain was transformed with HB1022(mrgA-lacZ)chromosomal DNA (1), generating strains HB14XX, or was transduced with SPβ085(mrgC-cat-lacZ), generating strains HB15XX. – See p. 8190, 2nd col., last paragraph.

And (see p. 8192, 1st col., 2nd and 3rd paragraphs):

> **Characterization of Trans-Acting Mutations**. To define factors involved in the regulation of mrgA, we have characterized 12 trans-acting mutants. Each mutant was cured of the SPβ1122 prophage, and transcriptional fusions to the mrgA (HB14XX series) and mrgC (HB15XX series) promoters were introduced. **All 12 HB14XX strains displayed increased mrgA-lacZ expression**, which was repressed little, if at all, by addition of Mn(II) (data not shown). We have described a second metalloregulated gene in B. subtilis, mrgC, which is repressed by iron but not by Mn(II) (1). In all 12 HB15XX strains, both mrgC-lacZ expression and the synthesis of catecholate siderophores were repressed normally by iron (data not shown). Therefore, regulation of mrgA expression [by both Mn(II) and iron] is independent of the postulated iron-dependent repressor, which regulates siderophore biosynthesis and mrgC expression.
>
> Like our trans-acting mutants, an H202R strain isolated previously, MA991 (8), is derepressed for mrgA expression. MA991 has a characteristic protein profile when analyzed by Coomassie-stained SDS/PAGE: MrgA, KatA, AhpC, and AhpF are all overproduced (8, 42). Nine of our trans-acting mutants shared this altered protein profile (data not shown) and are therefore likely to be mutant in the same regulatory pathway or even the same gene.

Therefore, the protein of interest can be the β-galactosidase, e.g. a heterologous protein, although it is apparent that said protein of interest can also be KatA, AhpC or AhpF as it is taught that these proteins are all over expressed as well - see above and also Figure 4, Group I strains and p. 8193, 1st col., 2nd para., "Like *mrgA, katA* is

induced by H_2O_2 during growth or by entry into stationary phase.", wherein Naclerio et al. clearly teach that KatA is a secreted enzyme and the MrgA protein is that which is known in the art, e.g. derived from strain MA991 (as taught above).

Applicants Remarks and Examiner's Rebuttal:

Applicants state that they believe the amended claims overcome the rejection of by Chen et al. but provide no rationale or reasoning in making these assertions.

The MrgA protein fused to LacZ are clearly overexpressed, said construct and said LacZ are furthermore heterologous. Since said LacZ or KatA is identified/construed as a protein of interest, the limitations of the claims have been met for a heterologous protein and secreted protein, respectively.

With regard to the sequence identities it is well known in the art that the MA991 strain is produced with, for example, the same protein as noted by above (Chen et al., 1995, Mol. Micro).

Conclusion

13. No claim is allowed.

14. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUZANNE NOAKES whose telephone number is (571)272-2924. The examiner can normally be reached on 7.00 AM-3.30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SUZANNE M. NOAKES/
Primary Examiner, Art Unit 1656
18 March 2011